BIRLA INSTITUTE OF TECHNOLOGY & SCIENCE, PILANI

First Semester 2022-2023 **Mid-Semester Examination**

Date: 04-Nov-2022	**BIO F311 Recombinant DNA Technology**	Max. Marks: 70 (35%)
Time: 3:00 to 4:30 PM	**(Closed Book)**	Duration: 90 min

The question paper is divided into 2 sections (A and B). Answer section A and B on separate sheets. Don't jumble up the order.

Section A

(*Answer all parts of this section on first sheet in the given order*)

Sheran is a molecular biologist. He is trying to make transgenic organisms. For this, he needs to first amplify and clone a foreign gene (gene of interest) into a vector to prepare the recombinant vector, transform the recombinant vector into bacteria, confirm cloning and then transform the recombinant plasmid into tomato plants. During the experiment, he faces several challenges, let us help him troubleshoot!

Q1. The first task would be to amplify the gene of interest using genomic DNA.

a) List the key features he needs to consider while designing the primers for a polymerase chain reaction (PCR).
b) Which factors/parameters of PCR experiment can impact the specificity and efficiency of the PCR reaction?
c) Can he use RT-PCR (Reverse transcriptase PCR) instead of conventional PCR to amplify the gene? Justify (no marks would be given without appropriate justification). **[3+3+2=8M]**

Q2. After PCR, he needs to confirm if he got the desired gene of interest amplified.

a) How can he determine if the PCR worked?
b) How can he determine if the amplified DNA corresponds to gene of interest and not a non-specific amplification? Justify. **[2+2=4M]**

Q3. Next step is to clone the gene of interest into a plasmid vector.

a) Describe any one method of cloning that he can use without using restriction enzymes.
b) What are the key features a plasmid must have to act as a cloning vector in the above step?
c) Which method can he use to transform bacteria with the recombinant plasmid. Illustrate the key steps in the form of a flow chart (only method needs to be illustrated) **[4+4+4=12M]**

Q4. Once bacteria are transformed, he would need to proceed with plasmid isolation.

a) Explain briefly a conformation-based method available for separating plasmid DNA from genomic DNA (only one method is required).
b) To determine if the plasmid extracted is free from genomic DNA he needs to perform gel electrophoresis. Which chemicals/reagents would be required for this technique? Provide the list (no need to mention plasticware, glassware and electrophoresis apparatus).
c) How can he determine the precise concentration of the plasmid? **[6+4+2=12M]**

Q5. While confirming the cloning:

a) How can he differentiate between bacteria containing self-ligated vector and the bacteria containing recombinant vector? Describe briefly.
b) How can he reduce the possibility of obtaining bacterial cells containing self-ligated vectors? **[4+4=8M]**

Q6. Describe the method he can use to transform tomato plants with the recombinant plasmid. Illustrate in the form of a flow chart (only one method is required). **[3M]**

Section B

(*Answer all parts of this section on second sheet in the given order*)

Q7. During a southern blotting procedure, transfer of a 10kb DNA fragment onto the nitrocellulose membrane, was seen to be very inefficient. Hypothesize on a pre-transfer procedural step which may have been omitted to result in the stated observation. Explain the logic behind this pre-transfer procedure. **[2+3M]**

Q8. Give a schematic flow chart of procedures, which could be used to detect a specific DNA band which has been separated on an agarose gel with the intention of using the DNA from that band for cloning purposes **[4M]**

Q9. Why would you use indirect autoradiography to detect hybridization signals for P^{32} labeled oligonucleotide probes? **[5M]**

Q10. Would washing of a membrane using 6X SSC be considered more stringent than washing with 4X SSC? Explain with the reasons. **[4M]**

Q11. What is the principle behind the procedure of Sangers technique for DNA Sequencing? **[5M]**

Good Luck!